U.S. PATENT APPLICATION

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Invention: TISSUE GRAFT

SPECIFICATION

TISSUE GRAFT

The present application claims priority from
Provisional Application No. 60/178,632, filed January
28, 2000, the entire content of which is incorporated
herein by reference.

TECHNICAL FIELD

The present invention relates to a method of
preparing a tissue graft material. The invention also
relates to a multipurpose tissue graft material and to
methods of using same as a replacement for vascular
and non-vascular tissue.

BACKGROUND

In general, biological tissues have a better
functional performance than equivalent synthetic
devices when used as a body implant. Tissue grafts
are presently largely limited to autologus and
allograft tissues that have inherent supply
constraints and logistic concerns of harvest,
transportation and serologies. Accordingly, there is
a need for additional sources of biological tissue
grafts. Animal tissues represent such a source.
Animal tissues can be relatively easily obtained from
slaughterhouses in large quantities. Prior to use,

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however, these tissues must be treated to remove antigenic proteins that elicit a rejection response by the host following implantation.

Removal of antigenic proteins can be achieved by processing the donor tissue in a manner such that the cellular component of the donor tissue is removed. Many antigenic proteins are present on cellular membranes. Therefore, removal of cells also removes these proteins. After decellularization, the tissue can be packaged and sterilized for use as a biological graft. Grafts can be implanted into humans and other animals to repair, augment or replace natural structures, systems or existing prosthetic devices. These include but are not limited to, cardiovascular, vascular, urogenital, neurological, gastrointestinal and orthopedic systems. Grafts can also be used to provide hemodialysis access.

The present invention provides a method of processing animal tissue so as to render it suitable for implantation into a human (or non-human) host. The invention also provides a method for processing human tissue for use as an allograft implant.

SUMMARY OF THE INVENTION

The present invention relates to a method of preparing a tissue graft material and to the resulting multipurpose graft material. The invention also

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relates to a method of using the tissue graft as a replacement for vascular or non-vascular tissue.

Objects and advantages of the present invention will be clear from the description that follows.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method of preparing an animal or human tissue in a manner so as to render it suitable for use in vascular and non-vascular graft applications. Tissue prepared in accordance with the method of the present invention exhibits physical and biological properties that render it particularly well adapted for tissue graft applications.

Tissue suitable for use in the present invention can be obtained from human cadavers or from bovine, porcine or other animal, for example, under abattoir conditions. Tissue can be transported to the point of tissue preparation under conditions necessary to keep the tissue at a desired temperature. Tissues can be transported, for example, immersed in a physiological salt solution and, upon arrival, inspected, washed, for example, in a physiological salt solution, and cleaned (dissected) free of unwanted adherent material, such as connective tissue and fat.

In a preferred embodiment, an isolated ureter is the tissue graft material. However, other tissues can

be used including arteries, veins, tendons, heart valves, fascia lata, pericardium and nerves.

After collection and dissection, the transplant tissue is advantageously, first washed, for example, with phosphate buffered saline (PBS), to reduce microbial bioburden. The tissue is then incubated (e.g., at about 37°C for about 18 hours) in a solution containing one or more antimicrobial agents, for example, an antibiotic or an antifungal agent, or mixture thereof, to further reduce the bioburden. Preferred antibiotics include amakacin, lincomycin, cefotaxime, vancomycin, rifampin, diflucan and amphotericin B. Advantageously, a mixture of these antibiotics is used. The tissue can then be cryopreserved for further processing at a later time or immediately subjected to decellularization.

Decellularization is preferably accomplished by incubating the tissue in a solution effective to lyse native cells in the tissue. Advantageously, the tissue is incubated (e.g., at about 37°C) in sterile water (for example, for about 4 hours in the case of ureters), however an aqueous hypotonic buffer or low ionic strength buffer can also be used. If desired, the decellularizing solution can include other agents, such as protease inhibitors ((e.g., chelators such as EDTA)).

After decellularization, the resulting tissue matrix is treated with an enzyme (e.g., nuclease)

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cocktail to degrade nuclear material. Nucleases that
can be used for digestion of native cell DNA and RNA
include both exonucleases and endonucleases. Other
nucleases are suitable for use in this step of the
process and are commercially available. For example,
a cocktail can be used comprising DNAse I (SIGMA
Chemical Company, St. Louis, Mo.) and RNAse A (SIGMA
Chemical Company, St. Louis, Mo.).

Preferably, the nucleases are present in a buffer
solution that contains magnesium and calcium salts
(e.g., chloride salts). The ionic concentration and
pH of the buffered solution, the treatment temperature
and the length of treatment are selected to assure the
desired level of effective nuclease activity. In the
case of ureters, the buffer is preferably a Tris
buffer at pH 7.6. Preferably, the nuclease cocktail
contains about 0.1 μg/ml to 50 μg/ml, preferably
17 μg/ml, of DNAse I, and about 0.1 μg/ml to 50 μg/ml,
preferably 17 μg/ml, of RNAse A. The nuclease
treatment can be effected at, for example, about 20°C
to about 38°C, preferably at about 37°C, for about 1
to 36 hours. In the case of ureters, nuclease
treatment for about 19 hours is typically sufficient.

Subsequent to decellularization and nuclease
treatment, the resultant tissue matrix can be treated
(washed) to assure removal of cell debris which may
include cellular protein, cellular lipids, and
cellular nucleic acid, as well as extracellular

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debris, such as extracellular soluble proteins, lipids
and proteoglycans. Removal of cellular and
extracellular debris reduces the likelihood of the
transplant tissue matrix eliciting an adverse immune
response from the recipient upon implant. For
example, the tissue can be incubated in a buffer
(e.g., PBS) or in a detergent solution such as a
solution of Triton X-100 in water. The composition of
the solution, and the conditions under which it is
applied to the tissue matrix can be selected to
diminish or eliminate the activity of the nuclease
utilized during nuclease processing and to remove cell
debris. The process can include incubation at a
temperature of between about 2°C and 42°C, with 37°C
being preferred. The tissue matrix can be incubated
in the detergent solution for up to 7 days, about
24 hours being sufficient in the case of a ureter
matrix. When buffer is used rather than a detergent
solution, the tissue matrix can be incubated for up to
30 days, about 14 days being sufficient in the case of
a ureter matrix.

If used, the detergent solution can be washed out
of the tissue matrix using multiple washes in a
sterile aqueous solution (e.g., water). Optimum wash
number and times can be readily determined, however,
about 4 30 minute washes are preferred in the case of
ureter matrices.

After washing, the tissue matrix can then be packaged, sterilized and/or stored prior to implantation. Advantageously, packaged tissue matrix is maintained in a non-frozen state, preferably at a temperature between 0°C and 40°C, more preferably, between 0°C and 20°C, most preferably between 2°C and 8°C up to and during sterilization using, for example, the approach used in Example 11. After sterilization, the tissue matrix can be maintained at room temperature. If desirable, the tissue matrix can be cryopreserved before or after sterilization for later use. Techniques of cryopreservation of tissue are well known in the art. Brockbank, K. G. M. Basic Principles of Viable TissuePreservation. In: Transplantation Techniques and use of Cryopreserverd Allograft Cardiac Valves and Vasular Tissue. D. R. Clarke (ed.), Adams Publishing Group, Ltd., Boston. pp 9-23, discusses cryopreservation of tissues and organs and is hereby incorporated by reference.

Tissue matrices of the invention, whether or not previously cryopreserved, can be sterilized using art recognized sterilization techniques. Advantageously, sterilization is effected using gamma irradiation at a dose of between 10kGy and 100kGy, preferably between 20kGy and 40kGy, more preferably between 25kGy and 40kGy. Alternative modes of sterilization include iodine peracetic acid treatment or electron beam. After sterilization, the tissue matrix can be stored

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frozen or unfrozen prior to implantation. If stored
cryopreserved, for example, in liquid nitrogen, the
tissue matrix is stable for at least 5 years. Prior
to using a frozen tissue matrix, the matrix is thawed
using a protocol designed to elute cryoprotectant
solutions. For example, the matrix can be thawed
rapidly to 4°C in a waterbath at a temperature of 37-
42°C. The matrix can then be quickly transferred to a
growth medium such as Dulbeccos' Modified Eagles
Medium (DMEM) containing mannitol (e.g., at about
0.5%). Mannitol and residual cryoprotectants can be
removed by serial dilution (washing) with 0.5, 0.25
and 0.0M solutions of mannitol in DMEM. Following the
washes, the tissue is ready to be used. For tissues
not stored frozen, alternate washing protocols can be
used, for example, washing in PBS or DMEM. Art-
recognized implantation procedures can be used and the
procedure selected is dependent on the tissue matrix
used and site of implantation.

The tissue matrix resulting from the above-
described process, particularly a ureter matrix, can
be used as a conduit (tubular) graft. For example, a
ureter matrix can be used as a vascular graft, nerve
guide, or replacement for any tubular structure,
including a ureter. When used as a conduit graft, the
diameter of the graft should generally be about the
same as the diameter of the native structure. The

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grafts of the invention demonstrate favorable characteristics as hemodialysis access grafts.

For use in other graft applications, a conduit graft (e.g., ureter tissue matrix) resulting from the above process can be cut longitudinally and rolled out to form a patch of tissue. The entire decellularization/nuclease treatment procedure described above can be carried out on patches of tissue (e.g., ureter tissue) prepared by cutting the segment longitudinally and unrolling it to form a pre-graft patch. The prepared graft patches can be utilized, for example, as a skin graft material or for repair of other body tissue defects lending themselves to surgical application of a tissue graft patch having the physical and functional characteristics of the present graft composition.

The tissue matrix of the present invention acts as a scaffold for spontaneous repopulation by host cells *in vivo* leading to tissue reconstruction and stabilization. The result is a fully functional, non-immunogenic, viable construct containing autologous cells expressing contractile proteins. The better patency rate and lack of infection seen in the present grafts may be attributable to the early incorporation, recellularization and remodeling of the matrix with host cells.

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Certain aspects of the present invention are described in greater detail in the non-limiting Examples that follow.

<div align="center">EXAMPLE 1</div>

<div align="center">Implantation of Decellularized Bovine Ureter as a
Peripheral Vascular Graft in the Dog</div>

Methods

Fresh bovine ureters were collected from slaughterhouses within 2 hours of death and shipped to the processing facility at 4°C in a solution of phosphate buffered saline (PBS) to prevent tissue degradation in transit. Upon receipt of the tissue, a gross tissue inspection was made and ureters of 4mm outside diameter and 30 to 40 cm in length were selected for processing. Selected ureters were dissected using sterile instruments to remove unwanted adherent material such as connective tissue and fat. Ureters were then placed in 300ml capcity polypropylene containers and washed four times with 250ml sterile PBS to reduce microbial bioburden. Ureters were then taken through steps to remove the tissue cell and antigen content.

Decellularization was initiated by incubation in a cocktail of antibiotics and antimycotic agents which consisted of a solution of antibiotics. This mixture contained the following: amakacin (34µg/ml), lincomycin (160µg/ml), cefotaxime (181µg/ml), vancomycin (136µ

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g/ml), rifampin (82μg/ml), diflucan (120μg/ml) and amphotericin B (0.5μg/ml). Incubation was for 18 hours in a shaking incubator at 37°C. For cell lysis, the antibiotic solution was replaced with 250ml of sterile water and incubation was allowed to proceed for 4 hours in a shaking waterbath at 37°C. This was followed by incubation for 19 hours at 37°C in an enzyme cocktail to degrade the nuclear material now exposed by lysing the cell organelles. This cocktail contained DNAse I (47 Kunitz U/ml) and RNAse A (1 Kunitz U/ml) in a solution containing magnesium chloride (1μg/ml)and calcium chloride (3μg/ml) and buffered using Tris[hydroxymethyl]aminomethane hydrochloride (50μg/ml) at pH 7.6. (The DNAse I and RNAse A were obtained from Sigma Chemical Company (D-5025 and R-5000) and both were used at a concentration of 17 μg/ml.) Subsequently, the tissue was placed in a 3.5mM solution of Triton X-100 detergent in sterile water to remove cell debris. This incubation was carried out for 24 hours at 37°C in a shaking waterbath. The detergent solution was then washed out of the tissue by four washes in sterile water at 37°C for 30 minutes each. After these washes, the resulting matrix was packaged, cryopreserved, sterilized and placed in storage prior to use as a vascular graft.

For preservation, the tissue was packaged in sterile packages containing Dulbeccos' Modified Eagles Medium

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solution (DMEM) and 10% dimethyl sulfoxide (DMSO) with 10% fetal bovine serum. Cryopreservation was performed using a controlled rate freezer to reduce the package temperature to -80°C at 0.5°C per minute. When the tissue temperature had reached -80°C each package was removed and placed in liquid nitrogen at -196°C for long term storage. Sterilization of tissue was performed in the frozen state using a 25-30kGy dose of gamma radiation. Following sterilization, the tissue was stored at -196°C in liquid nitrogen until use.

Prior to implantation, the tissue matrix was thawed to remove the cryoprotectant solution from the tissue. The grafts were thawed rapidly to 4°C in a waterbath at a temperature of 37-42°C. Tissue was quickly transferred to DMEM containing 0.5% mannitol. Mannitol and residual cryoprotectants were removed by dilution with 0.5, 0.25 and 0.0M solutions of mannitol in DMEM. Following these washes, implantation of acellular bovine conduit (that is, the resulting tubular tissue matrix) was performed as an end-to-side interpositional graft in the left and right carotid and left femoral arteries of an adult mongrel dog. The graft lengths were between 9 and 12 cm and the internal diameter of the grafts was between 5 and 10 mm. Implantation was made using standard surgical techniques for vascular graft implantation in these positions. An oral anticoagulant regimen of 325mg

Aspirin and 75mg Persantin was administered daily to the animal beginning two days prior to the surgical procedure.

At two weeks and at four weeks after surgery, arteriograms were performed to determine the patency of the implanted grafts. The animal was sacrificed and the grafts explanted immediately following the second arteriogram at four weeks after the surgery. Explanted grafts were evaluated for patency and gross appearance and further examined histologically to determine graft microscopic integrity.

Results

During the four weeks duration of the study, the animal behaved normally and there were no complications following surgery. At two weeks and at four weeks after surgery, all three bovine ureter grafts were determined to be fully patent on angiographic examination. At four weeks after surgery, gross analysis of the explanted graft tissue indicated there to have been a healing response that had stabilized the grafts into the surgical site and the patency of all the grafts was confirmed by observing flow through the graft prior to placing the grafts in formalin for fixation. After fixation each graft was cut into seven separate samples for histological analysis. Samples were taken from the native artery at both proximal and distal ends away

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from the graft. Sections of the proximal and distal
anastamosis sites were taken along with the proximal,
middle and distal portion of each graft. Following
processing, paraffin embedding and sectioning, graft
samples were stained using a standard hematoxylin and
eosin stain. Microscopic analysis revealed the grafts
to be structurally intact. The matrix of the bovine
ureter had begun to become re-vitalized through the
movement of cellular components from the outer edges
of the surgical area. Through this remodeling the
grafts were taking on the appearance of natural
arterial blood carrying conduits.

<center>EXAMPLE 2</center>

<center>Implantation of Decellularized Porcine Ureter
as a Peripheral Vascular Graft in the Dog</center>

Methods

Porcine ureter tissue was collected and prepared
and preserved exactly as described in Example 1 with
the exception that the dimensions of tissues selected
for processing were 3mm in internal diameter and 25 cm
in length. Implantation of treated porcine ureters
was made in an adult mongrel dog as an end-to-end
interpositional vascular graft in the left and right
femoral arteries and in the left and right carotid
arteries. All grafts were examined by arteriogram two
weeks after surgery. The animal was sacrificed four
weeks after surgery and the grafts were explanted for

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gross examination and histological evaluation of
performance. Histology samples were taken and stained
as described in Example 1.

Results

Arteriograms performed two weeks after
implantation showed the grafts to be patent. On
explantation, gross examination indicated the grafts
to be fully patent and difficult to distinguish from
the native blood vessel. Histological examination
showed the grafts to have become partly recellularized
with spindle-shaped cells. This recellularization
likely represents the first stages of remodeling into
a fully-functional blood-carrying conduit that would
be indiscernible from native tissue.

EXAMPLE 3

Implantation of Decellularized Bovine Uterine Artery
as a Peripheral Vascular Graft in the Dog

Methods

Bovine uterine artery tissue was collected and
prepared and preserved exactly as described in Example
1. Implantation of treated bovine uterine artery was
made in an adult mongrel dog as an end to side
interpositional graft in the carotid artery. After 4
weeks of implantation, the grafts were explanted and
taken for histological analysis.

Results

At four weeks after implantation, the grafts were patent. Histology indicated these grafts to have begun to take on cells from the host animal.

EXAMPLE 4

Implantation of Decellularized Bovine Gastric Artery
as a Peripheral Vascular Graft in the Dog

Methods

Bovine gastric artery tissue was collected and prepared and preserved exactly as described in Example 1. Implantation of treated bovine uterine artery was made in an adult mongrel dog as an end to side interpositional graft in the carotid artery. After 4 weeks of implantation, the grafts were explanted and taken for histological analysis.

Results

At four weeks after implantation, the grafts were patent. Histology indicated these grafts to have begun to take on cells from the host animal.

EXAMPLE 5

Determination of Burst Strength Characteristics
of Decellularized Bovine Ureter

Methods

Three segments of bovine ureter tissue were collected and prepared and preserved exactly as described in Example 1. Using compressed nitrogen

gas, the pressure required to burst the graft was
determined by slowly increasing the head-pressure of
nitrogen applied to the graft. The gas was contained
within the graft using standard high-pressure pipe
fittings and cable ties. Each graft segment was
tested in duplicate and average burst strength for
each graft was calculated.

Results

The three grafts were found to burst at 3361,
2456 and 2327mm Hg, the average being 2715mm Hg. This
magnitude represents a burst strength of around 1.5
times that of the human fresh saphenous vein which is
commonly used in bypass surgical procedures.

EXAMPLE 6

Determination of Burst Strength Characteristics
of Decellularized Porcine Ureter

Methods

Six segments of porcine ureter tissue were
collected and prepared and preserved exactly as
described in Example 2. Using compressed nitrogen
gas, the pressure required to burst the graft was
determined by slowly increasing the head-pressure of
nitrogen applied to the graft. The gas was contained
within the graft using standard high-pressure pipe
fittings and cable ties. Each graft segment was
tested in duplicate and the average burst strength for
each graft was determined.

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Results

The six grafts were found to burst at 2068, 5171, 6722, 6722, 5688 and 3620mm Hg, the average being 4999mm Hg. This value represents a burst strength of almost 3 times that of human fresh saphenous vein which is commonly used in bypass surgical procedures.

EXAMPLE 7

In Vitro Recellularization of Decellularized Bovine Ureter with Vascular Conduit Cells

Methods

Three segments of bovine ureter tissue were collected and prepared and preserved exactly as described in Example 1. Each piece of tissue was placed into a 75cc tissue culture flask containing DMEM and supplemented with 10% fetal bovine serum. Each graft was seeded with endothelial cells or smooth muscle cells to enable cell growth into the graft segments. Cultures were fed using fresh serum-supplemented DMEM two times each week for 4 weeks. After 4 weeks, the tissues were extracted from the culture system and examined using histological sectioning of the tissue and H&E staining.

Results

After four weeks of cell culture, endothelial cells were observed growing on the surface of graft tissue but not internally. In addition, smooth muscle cells were found growing on the surface of the grafts

and in the wall of the graft material once they could
gain access on the surface of the graft.

EXAMPLE 8

Tissue Graft Derived from Ureter
as Aortic Graft in the Dog

Methods

Bovine ureters were used to provide the conduit
matrix for the vascular tissue graft. These tissues
are available in lengths and diameters suitable for a
number of vascular applications and they do not
contain valves in the lumen or possess tributaries
that require ligation. Ureters were obtained from U.S
Department of Agriculture approved slaughterhouses.
The tissues were washed in physiological salt solution
and transported for tissue preparation on ice within
24 hours of harvest. Ureters were first dissected
free of adherent connective tissue and fat and only
segments with a 6mm internal diameter were taken for
further processing.

Initial processing consisted of bioburden
reduction using a solution of multiple antibiotics as
described in Example 1. Removal of greater than 95% of
all cellular material was achieved in several steps.
First, incubation in sterile water produced hypotonic
cell lysis. The resulting tissue matrix was then
equilibrated in buffer (PBS) and treated with a
solution containing ribonuclease and deoxyribonuclease

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(see Example 1). An isotonic washout over several days completed the cellular protein removal. Removal of cellular debris was monitored using hematoxylin and eosin staining of histological sections. Tissue matrices were then sterilized by gamma irradiation (25kGy to 40kGy) prior to use and analysis of sterility was carried out on each processing batch.

Eight mongrel dogs weighing 50 to 60 lb were anesthetized with sodium thiopental, endotracheally intubated and placed on inhaled isoflurane. The abdomens were prepared and draped in sterile fashion. A midline incision was made and the abdominal aorta distal to the renal arteries was isolated in each dog. Vascular conduits were prepared by washing the tissue matrix in 100ml of sterile HEPES-buffered Dulbecco's Modified Eagle Medium and a segment approximately 6cm in length and 6mm in internal diameter was inserted as an aortic interposition graft using interrupted prolene sutures to construct proximal and distal, end to end, anastomoses. All animals received 325mg aspirin and 75mg dipyridamole p.o. daily for 2 days prior to, and for 14 days following, surgery.

Patency and structural stability were observed with angiographic examination following surgery every 6 weeks in the longer survivors and once immediately prior to euthanasia. Two animals were sacrificed at 3 weeks, 3 at 6 weeks, and 1 animal at 13 weeks after surgery. The 2 remaining animals were last evaluated

at 43 weeks and are still living. After sacrifice,
grafts were removed in bloc incorporating proximal and
distal anastamoses inspected grossly and processed for
histological analysis.

Following harvest, grafts were fixed in 10%
buffered formaldehyde solution. The whole of the graft
along with anastamotic sites and proximal and distal
native aorta was divided into 7 tissue segments and
placed in paraffin blocks for processing. Hematoxylin
and eosin-stained sections of these tissues were
examined and immunohistochemical analysis was carried
out using specific antibiotics to identify the
presence of smooth muscle α-actin (α-SMA), desmin and
vimentin contractile filaments.

Results

After processing, vascular tissue grafts prepared
from bovine ureter showed removal of greater than 95%
of bovine cellular material. The remainder consisted
of cellular debris and not intact cells. Conduit
graft sterility and pyrogen levels of below 20
endotoxin units were demonstrated. Implantation of
these interposition grafts into the canine infrarenal
aorta was uncomplicated and handling properties of the
grafts were similar to normal vascular tissue.

Arteriograms performed on each of the dogs
indicated grafts to be fully functional over the 43-
week implant period without the appearance of dilation

or stenosis. Gross evaluation of all explanted grafts after 3, 6 and 13-weeks of implantation confirmed fully patent grafts. Histologic examination showed a healing response around the graft adventitia with recellularization of the media. A layer of cells on the lumenal surface resembled endothelium. All cells found in the graft were presumed to have originated from the host because the original graft material was acellular. The extent of medial recellularization was approximately 20% at 3-weeks, 30% at 6-weeks and 50% at 13-weeks. Revitalization of the graft media appeared to occur from the adventitial area towards the lumen and as recellularization progressed, there was circumferential organization of cells growing perpendicular to the flow of blood in the conduit.

Analysis of anastomotic sites showed intimal hyperplasia to be minimal and cellular overgrowth was evident at the suture-line creating a smooth transition from native aorta to graft. Histologically, there was no evidence of hyperplastic reaction narrowing the lumen in the graft explanted after 13-weeks. Also, narrowing was not observed angiographically up to 43-weeks after implantation.

Immunohistochemistry staining was used to identify the type of cells present in the recellularized grafts. The proportion of cells expressing smooth muscle contractile proteins were demonstrated using stains containing antibodies to α-

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SMA, desmin and vimentin. A very large percentage of
medial cells at 3, 6 and 13 weeks, were α-SMA positive.
Vimentin was also commonly expressed by α-SMA positive
cells. Desmin positive cells were less abundant but
present in a sub-population. Most of the cells
present in the grafts stained positive for at least
one of these contractile proteins. As no intact cells
were present in the graft conduits prior to implant,
all cell-specific immunostaining demonstable for α-SMA,
desmin and vimentin was present on cells that had
originated from the host.

<center>EXAMPLE 9</center>

<center>Use of Tissue Graft Derived from Ureter
as an Arterio-Venous Fistula in the Dog</center>

Methods

Nine segments of treated bovine ureter tissue
graft conduit, prepared as described in Example 9,
(20cm x 6mm ID) were implanted as arteriovenous grafts
in the carotid artery (CA) and jugular vein (JV)
(n=5), or in the femoral artery (FA) and femoral vein
(FV) (n=4) in 6 adult dogs. A control group of 7 dogs
received 11 (6mm ID) polytetrafluoroethylene (PTFE)
grafts (7 in the CA and JV, and 4 in the FA and FV).
All grafts were matured for 14 days and then sham-
accessed once weekly with two 17-gauge hemodialysis
needles. Routinely over a 6-month period, patency was

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assessed and blood was drawn to monitor CBC and clotting factors. Histological analysis was performed in a sub-group of explanted grafts at 2, 4, 10 and 24 weeks.

Results

27% (3/11) of the PTFE grafts became infected, while none of the tissue graft conduits prepared in accordance with the present invention became infected during the study. The patency rate of the tissue graft conduit was 86% compared to 72% for the PTFE grafts. The white blood cell count was not elevated in either group at 2 and 7 weeks and blood clotting factors were also unchanged. The hemostasis times after sham sticking of the grafts was longer (mean 10 minutes) in the PTFE grafts compared to the tissue graft conduits (mean 3 minutes). Histology at 10-weeks showed tissue graft conduits to have undergone recellularization of the tunica media with host spindle shaped cells as well as excellent incorporation into surrounding tissues as evidenced by capillary ingrowth into the tunica adventitia. PTFE grafts showed no significant cellular ingrowth and an absence of luminal endothelium.

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EXAMPLE 10

Packaging and Sterilization of Tissue Graft

Packaging

Tissue product is packaged in heat sealed clear
polyester pouches containing phosphate buffered saline
and stored at 4° centegrade for up to 7 days prior to
sterilization.

Shipping

Three frozen 2lb cold gel bricks are placed in
the bottom of a pre-chilled 19" x 14.5" x 22"
cardboard container insulated with 2" polyurethane
foam. Two cardboard separators are placed on top of
these.

Three cold 2lb gel bricks are added followed by
product load (1,100 cubic inches).

Two filler bags are used as temperature
indicators on which various temperature indicator
strips are present, the filler bags are placed among
the samples. A seven-day mechanical temperature
recorder is also placed among the samples.

Three cold 2lb gel bricks are place on top of the
product load followed by a cardboard separator and
three frozen 2lb gel bricks. A foam plug is placed on
top of the last layer of bricks and the box is closed.

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The box is shipped to sterilization facility for sterilization by gamma irradiation at 25-40 kGy. The box is then returned and product is unpacked and stored at room temperature until use. The total tissue time between packing and unpacking is advantageously less than 100 hours and the temperature is maintained throughout this period at 2°C-8°C.

Storage

The tissue graft can be stored at room temperature for 2 years.

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All documents cited above are hereby incorporated in their entirety by reference.

One skilled in the art will appreciate from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention.